

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

① **BLACK BORDERS**

- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61L 25/00, A61K 35/14	A1	(11) International Publication Number: WO 97/40864 (43) International Publication Date: 6 November 1997 (06.11.97)
(21) International Application Number: PCT/US97/08472 (22) International Filing Date: 30 April 1997 (30.04.97) (30) Priority Data: 08/640,278 30 April 1996 (30.04.96) US (71) Applicant: MEDTRONIC, INC. [US/US]; 7000 Central Avenue, Minneapolis, MN 55432 (US). (72) Inventor: BAUGH, Robert, F.; 7926 East Windcrest Row, Parker, CO 80134 (US). (74) Agents: PETERSEN, Steven, C. et al.; Chrisman, Bynum & Johnson, P.C., 1900 Fifteenth Street, Boulder, CO 80301 (US).		(81) Designated States: DE, JP. Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHOD FOR MAKING AUTOLOGOUS FIBRIN SEALANT (57) Abstract A method of producing a fibrin sealant. Platelet rich blood plasma and recombinant thromboplastin are mixed to effect the formation of fibrin sealant.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

METHOD FOR MAKING AUTOLOGOUS FIBRIN SEALANT**Description****Technical Field**

The present invention relates to the preparation of fibrin based sealants.

Background Art

The preparation and use of fibrin based sealants is becoming more prevalent in medical practice. This is due to the biocompatibility of such sealants.

Biocompatibility has some significant issues, however, when the present methods of forming the sealants are examined. The most common method is to use what is known as bovine thrombin preparations. These type of preparations have been approved for medical use for several years; however, recent findings suggest that there are significant problems associated with their use. These problems include: 1) the risk of transmission of bovine spongiform encephalitis, and 2) the development of immune responses to the thrombin and contaminants in the thrombin which cause the development of autoimmune antibodies to various human coagulation factors. This results in patients who develop pseudohemophilia and are at increased risk for developing severe bleeding problems.

Human recombinant thromboplastin is presently available as a diagnostic reagent for use in performing various coagulation assays.

Fibrin sealants are made from several different types of starting materials, including: 1) citrated plasma, 2) concentrated citrated plasma, 3) platelet rich citrated plasma, 4) cryoprecipitates, and 5) purified plasma fractions which contain high quantities of fibrinogen. The coagulation of blood is a rather complex process. The primary reaction of producing a clot is caused by the action of thrombin on the fibrinogen molecule which converts fibrinogen to fibrin. Fibrin spontaneously polymerizes, forming a net-like structure. This structure is later solidified and enhanced by the actions of several other factors in blood, which factors are also generated by the action of thrombin. Most of the factors found in the blood are in an inactive form. Thrombin has an inactive form, prothrombin. Thus at some point there must be a trigger for the initiation of blood clotting. One of these mechanisms is thromboplastin. When blood or blood plasma is exposed to thromboplastin, it triggers the activation of these factors which leads to the generation of thrombin which in turn converts fibrinogen to fibrin.

Disclosure of Invention

It is the principal object of the present invention to provide an improved method of producing fibrin sealants or adhesives.

It is a further object of the present invention to provide an improved method of the foregoing character for producing fibrin sealants or adhesives wherein the risk of transmission of bovine disease and virile human disease which would be associated with the use of human thrombins purified from heterologous sources is substantially reduced or eliminated.

The present invention is embodied in a method for making a fibrin sealant adhesive or glue which does not introduce either immunologic or viral concerns. To this end, fibrin sealant is produced by utilizing human recombinant thromboplastin.

Best Mode for Carrying out the Invention

5 In accordance with the foregoing objects, the present invention is embodied in a method wherein human recombinant thromboplastin is mixed directly with the precursor of the fibrin sealant such as blood plasma, platelet rich blood plasma, concentrated blood plasma or cryoprecipitate. Alternatively, human recombinant thromboplastin is utilized to generate thrombin in a small aliquot of plasma or the supernatant from a cryoprecipitation, and then the thrombin
10 thereby generated is combined with the precursor of the fibrin sealant. Both procedures produce a fibrin sealant which can then be used in the conventional manner.

METHOD 1

Fibrin Sealant Source: Platelet Rich Plasma

The platelet rich plasma is collected into a 1:9 volume of 3.8% sodium citrate. Low speed
15 centrifugation leads to the production of the platelet rich plasma. To form the sealant, the platelet rich plasma is mixed with recombinant thromboplastin in a suitable container and sufficient calcium chloride is added to neutralize the citrate used as the anticoagulant. The ratios of recombinant thromboplastin, calcium, and platelet rich plasma are preferably determined in small test tubes. The desired result is to effect the formation of a fibrin sealant gel in one to two minutes after the
20 combination of the above agents.

METHOD 2

Fibrin Sealant Source: Platelet Rich Plasma

Plasma Source for Thrombin: Prepared by High Speed Centrifugation

Blood plasma, citrated as described above, is mixed with recombinant thromboplastin and
25 calcium. The resulting clot is agitated to break up the clot. The supernatant fluid, which contains thrombin, is separated by centrifugation. The thrombin is then used as in Method 1 to generate a fibrin sealant from the platelet rich plasma.

Claims

1. A method of producing a fibrin sealant comprising mixing platelet rich blood plasma and recombinant thromboplastic to effect the formation of fibrin sealant.
2. A method of producing a fibrin sealant comprising mixing citrated blood plasma, recombinant thromboplastin and calcium, separating thrombin from said mixture, and adding said thrombin to platelet rich plasma to generate fibrin sealant.

INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/US 97/08472

 A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 A61L25/00 A61K35/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

 Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US SUZUKI M. ET AL.: "CLINICAL APPLICATION OF THE FIBRIN ADHESIVE" XP002040697 see abstract & OTOLARYNGOLOGY, vol. 56, no. 11, 1984, TOKYO, pages 949-953, ---	1,2
E	WO 97 29792 A (COHESION CORP ;SIERRA DAVID H (US)) 21 August 1997 see the whole document --- -/--	1,2

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *Z* document member of the same patent family

Date of the actual completion of the international search

15 September 1997

Date of mailing of the international search report

30-09-1997

Name and mailing address of the ISA

 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+31-70) 340-3016

Authorized officer

ESPINOSA, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/08472

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 443 724 A (BAXTER INT) 28 August 1991 see column 1, line 41 - line 54 see column 5, line 51 - line 58; claims ---	1,2
A	FR 2 696 095 A (INOTEB) 1 April 1994 see claims; examples -----	1,2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PLI/US 97/08472

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9729792 A	21-08-97	NONE	
EP 0443724 A	28-08-91	JP 5227962 A US 5354682 A	07-09-93 11-10-94
FR 2696095 A	01-04-94	EP 0615454 A WO 9407548 A JP 7505076 T US 5589462 A	21-09-94 14-04-94 08-06-95 31-12-96